Evidence on the effect of uncontrolled diabetes mellitus on orthodontic tooth movement. A systematic review with meta-analyses in pre-clinical in- vivo research

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Abstract: Objective The aim of this review was to appraise the existing evidence from pre- clinical research on tooth movement under the condition of hyperglycemic status. Design Electronic search was conducted in 8 databases in October 13, 2019, to identify related pre- clinical animal research with keywords being: "diabetes mellitus", "tooth movement". Eligibility criteria involved controlled animal studies, entailing tooth movement under diabetic status compared to control healthy animals. Primary endpoints involved all outcomes related to tooth movement. Risk of bias (RoB) was assessed through the SYstematic Review Centre for Laboratory animal Experimentation tool (SYRCLE), while quantitative synthesis was planned after exploration of heterogeneity, through random effects meta-analyses of standardized mean differences (SMDs) with 95 % confidence intervals (CIs). Results Of an initial number of 290 articles retrieved, 14 papers were eligible for inclusion in the qualitative synthesis, while 9 contributed to meta-analyses. Heterogeneity of experimental conditions in individual studies was evident. The risk of bias overall was rated as unclear to high. There was no evidence of a significant effect of diabetes mellitus when tooth movement was assessed macroscopically (6 studies, SMD: 1.47; 95 % CI: -0.60, 3.53; p = 0.16). However, attenuation of osteoblastic differentiation within the periodontal ligament was detected, as there was evidence of reduction of osteopontin expression (2 studies, SMD: -3.77; 95 %CI: -4.89, -2.66; p < 0.001). Conclusions There is currently a paucity of solid evidence with regard to alterations of the equilibrium of the implicated structures under the status of diabetes mellitus, when mechanical stimulation of teeth is attempted, with sporadic inferences from animal research. Significant research insights in how the disease impacts on orthodontic tooth movement are invaluable, at present.

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Evidence on the effect of uncontrolled diabetes mellitus on orthodontic tooth movement. A systematic review with meta-analyses in pre-clinical *in- vivo* research

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Structured Abstract

Objective: The aim of this review was to appraise the existing evidence from pre- clinical research on tooth movement under the condition of hyperglycemic status.

Design: Electronic search was conducted in 8 databases in October 13, 2019, to identify related pre-clinical animal research with keywords being: “diabetes mellitus”, “tooth movement”. Eligibility criteria involved controlled animal studies, entailing tooth movement under diabetic status compared to control healthy animals. Primary endpoints involved all outcomes related to tooth movement. Risk of bias (RoB) was assessed through the SYstematic Review Centre for Laboratory animal Experimentation tool (SYRCLE), while quantitative synthesis was planned after exploration of heterogeneity, through random effects meta-analyses of standardized mean differences (SMDs) with 95% confidence intervals (CIs).

Results: Of an initial number of 290 articles retrieved, 14 papers were eligible for inclusion in the qualitative synthesis, while 9 contributed to meta-analyses. Heterogeneity of experimental conditions in individual studies was evident. The risk of bias overall was rated as unclear to high. There was no evidence of a significant effect of diabetes mellitus when tooth movement was assessed macroscopically (6 studies, SMD: 1.47; 95% CI: -0.60, 3.53; p=0.16). However, attenuation of osteoblastic differentiation within the periodontal ligament was detected, as there was evidence of reduction of osteopontin expression (2 studies, SMD: -3.77; 95%CI: -4.89, -2.66; p<0.001).

Conclusions: There is currently a paucity of solid evidence with regard to alterations of the equilibrium of the implicated structures under the status of diabetes mellitus, when mechanical stimulation of teeth is attempted, with sporadic inferences from animal research. Significant research insights in how the disease impacts on orthodontic tooth movement are invaluable, at present.

Keywords: diabetes mellitus, hyperglycemia, tooth movement, periodontal ligament, orthodontic, systematic review

Introduction

Diabetes Mellitus (DM) is a common metabolic disorder, characterized by deficiency of insulin secretion or action, leading to chronic hyperglycemia and disturbances of carbohydrate, fat, and protein metabolism (Chau, Edelman, & Chandran, 2003; Verhaeghe et al., 1989). Type 1 DM is caused by an absence of insulin secretion, which results from an autoimmune destruction of pancreatic beta cells, and accounts for 5- 10% of diabetic patients (Bensch, Braem, Van Acker, & Willems, 2003; Burden, Mullaly, & Sandler, 2001). Type 2 DM accounts for 90- 95% of diabetic patients, representing variable degrees of insulin deficiency/ resistance (American Diabetes Association, 2009).

The broader orthopedics literature demonstrates that diseases presenting connective tissue manifestations such as Type 1 DM, demonstrate collagen degenerative processes affecting the tendons and/ or ligaments as well as alterations in bone metabolism (He et al., 2004; Liu et al., 2006). Orthodontic tooth movement comprises of multiple biological processes characterized by consecutive reactions of the periodontal tissue in response to biomechanical forces. During orthodontic tooth movement, a concurrent compression of the matrix takes place in the pressure site along with a tension in the opposite site, resulting in alveolar bone resorption and apposition respectively (Jónsdóttir, Giesen, & Maltha, 2006; K. Papadopoulou et al., 2013). During tension the load is distributed to the collagen bundles and thus any reduction in their properties arising from structural defects, might bear unfavorable consequences for the integrity of the tissue (Jónsdóttir et al., 2006; K. Papadopoulou et al., 2013). Moreover, the mechanical stress or stretching generated during orthodontic movement causes controlled amounts of tissue injury that triggers the activation of inflammatory mediators, resulting in a completely new state of periodontal homeostasis. Therefore, inflammation is inbred when orthodontic induced tooth movement takes place.

The presence of diseases or conditions that modify inflammatory response, such as DM, may also change the host's response to orthodontic force (Villarino, Lewicki, & Ubios, 2011). Due to the attenuated osteoblastic activity and the enhanced apoptosis of osteoblast cells, imbalances between bone apposition and resorption might be present in diabetic patients during orthodontic treatment (Najeeb et al., 2017). Furthermore, evidence suggests significant alterations in bone density in diabetic patients, even in patients under control with intensive insulin treatment

(Guarneri, Weber, Gallia, & Chiumello, 1993; Hofbauer, Brueck, Singh, & Dobnig, 2007). When diabetes is uncontrolled or poorly controlled, severe degradation of periodontal tissues may occur, therefore contraindicating orthodontic treatment until the metabolic disorder is compensated (Bensch et al., 2003; Burden et al., 2001).

A systematic review from 2017 (Najeeb et al., 2017) was the sole source of synthesized evidence on the effects of uncontrolled diabetes mellitus on tooth movement pertaining to orthodontic treatment. However, this review included a limited number of individual studies, did not include a mathematical synthesis, ie. a meta-analysis, that would allow for a more precise estimation of the overall treatment effect, and focused on the rate and magnitude of tooth movement as an outcome of interest. Effectively, there is a timely need for a more complete approximation of the mechanisms underlying the effect of distorted metabolic status on the periodontal apparatus and mechanical stimulation resulting in tooth movement. As such, the aim of the present systematic review was to collect, synthesize and appraise existing evidence, from pre- clinical in vivo research, on any documented quantitative outcome related to tooth movement under the condition of hyperglycemic status. The null hypothesis of this research was that there is no difference in the effect of hyperglycemic or normoglycemic status on orthodontically induced tooth movement.

Materials and Methods

Protocol Registration and Reporting

The protocol for this work has been designed *a priori* and has been registered at the Open Science Framework (https://osf.io/f6ga4/) as of October 13, 2019. This systematic review was conducted according to the Cochrane Handbook (*Cochrane Handbook for Systematic Reviews of Interventions*, 2019) and followed the reporting schemes of the PRISMA statement (Liberati et al., 2009; Moher, Liberati, Tetzlaff, & Altman, 2009).

Eligibility Criteria

The following inclusion criteria for study selection were applied in line with the Participants, Intervention, Comparator, Outcomes (PICO) guidance and modified accordingly for animal research:

- Study Design: randomized or non-randomized experimental in- vivo studies involving animals and including a comparison group will be considered (one or more comparators)
- Population: all animal models undergoing any type of orthodontic tooth movement or mechanical stress in the periodontal ligament are considered eligible, of both gender, at any age, or species. On an exploratory basis, insulin/ other medication treated animals were considered as well.
- Intervention: tooth movement, or periodontal ligament mechanical stimulation in diabetic animals.
- Comparator: tooth movement, or periodontal ligament mechanical stimulation in control healthy animals, with no underlying diabetes mellitus.
- Outcomes (primary): Any quantitative outcome related to orthodontic tooth movement or stimulation of the periodontal ligament, including but not confined to: rate of tooth movement, root resorption, expression of inflammatory mediators, expression of genes related to osteoblastic/ osteoclastic differentiation, bone loss, osteoclast cells.

Exclusion Criteria:

- Animal studies with no comparison group
- Animal studies involving administration of pharmaceutical/exogenous hormones/molecules to populations
- Any outcome not related to tooth movement

Search Strategy

Electronic search was formulated and conducted by one author (DK) in eight databases for eligible published and unpublished research items. No language or chronologic restrictions were applied to the search. Specifically, the search was employed within: Medline via Pubmed, Scopus, Cochrane Central Register of Controlled Trials (CENTRAL), Cochrane Database of Systematic Reviews (CDSR), Open Grey, ClinicalTrials.gov (www.clinicaltrials.gov), National Research Register (ISRCTN: www.controlled-trials.com), PERGAMOS (pergamos.lib.uoa.gr, online repository of research theses and dissertations of the National and Kapodistrian University of Athens). In addition, hand search of the retrieved for full text evaluation articles was performed for any potential for inclusion publication. Access was sought in October 13, 2019 (Appendix A). Representative keywords included “tooth movement”, “diabetes mellitus”, “orthodontic movement”. Initial screening for

eligible articles was done by one reviewer (DK) and confirmed by a second (AI), with documented disagreements being resolved after consultation with a third reviewer (TE), who settled potential discrepancies.

Data Extraction

Data extraction was performed independently by one reviewer (AI), while confirmed by a second (DK) and all relevant information was extracted on standardized piloted forms. Initial piloting for data extraction was done in 30 percent of the included articles. Specifically, information on year of publication, origin, sample size, interventions/ comparators, outcomes, method of outcome assessment, timescale for the application of orthodontic force, as well as protocol for diabetes induction in animals, was recorded. Both reviewers were not blinded on study title and authorship.

Risk of Bias Assessment in Individual Studies

Risk of bias (RoB) in individual studies was assessed with the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) RoB tool for animal studies (Hooijmans et al., 2014). In particular, the tool comprises of the following principal domains which were were considered: 1. Sequence generation, 2. Baseline characteristics, 3. Allocation concealment, 4. Random housing, 5. Blinding of researchers, 6. Random outcome assessment, 7. Blinding of outcome assessors, 8. Incomplete outcome data, 9. Selective outcome reporting, 10. Other sources of bias.

An overall assessment of the risk of bias was made for each included study (high, unclear, low). Studies with at least 1 item designated to be at high risk of bias were regarded as having an overall high risk of bias. Reports with unclear risk of bias for one or more key domains were considered to be at unclear risk of bias and likewise, studies with low risk of bias in all domains were rated as low risk of bias.

Overall risk of bias assessment was done by one author (DK) after calibration with a second (AI) on 30 percent of the articles under consideration. Ratings were confirmed by the second author, while in cases of disagreements, a third author (TE) was consulted to settle down discrepancies.

Summary Measures and Data Synthesis

A quantitative synthesis was planned for all outcomes given the availability of the retrieved studies as well as the homogeneity on key domains. Clinical heterogeneity of the eligible for inclusion studies was assessed through the examination of study design/ settings, eligibility criteria, populations, interventions, experimental conditions and data collection methods. Statistical

heterogeneity was examined through visual inspection of the confidence intervals (CIs) for the treatment effects on forest plots. A formal χ^2 test was also applied to assess heterogeneity; a P value below the level of 10% ($P < 0.1$) is considered indicative of significant heterogeneity I^2 (Higgins, Tompson, Deeks, & Altman 2003). I^2 test for homogeneity was undertaken as well. As continuous outcomes were expected to be explored, values for each specific outcome were calculated through standardized mean differences (SMDs) with associated 95% Confidence Intervals (95% CIs). Random effects meta-analyses were conducted as they were considered more appropriate to reflect the expected heterogeneity and variations in laboratory settings, experimental conditions and variations in tooth movement related parameters.

Risk of Bias Assessment Across Studies

If more than ten studies were included in the meta-analyses, publication bias and small study effects were investigated through standard funnel plots (Sterne, Egger, & Moher, 2011; Sterne et al., 2019) and Egger's regression test (Egger, Smith, Schneider, & Minder, 1997).

Additional Analyses

Sensitivity analyses were preplanned to explore the effect of studies with high risk of bias and isolate the effect of lower risk of bias studies to the overall pooled estimate, in case both high and lower risk of bias studies being included in the quantitative synthesis.

In addition, when extremely high or low variability of relevant estimates were recorded in individual studies, then, sensitivity analyses were conducted to isolate and remove this effect.

All analyses were undertaken in Review Manager (RevMan 5.3) software (*Review Manager (RevMan) [Computer program]*, 2014).

Results

Search Details

After electronic database and hand- searching, a total of 290 eligible articles were retrieved, with 19 papers left for full- text evaluation after duplicate removal, title and abstract screening. Finally, five articles were excluded for reasons related to our pre- defined eligibility criteria, leaving a number of 14 articles for inclusion in this systematic review (Figure 1) (Arita et al., 2016; Braga et

al., 2011; Damanakis, 2018; Ferreira et al., 2018; Gomes et al., 2017, 2018; Li, Zhang, Wang, Feng, & Bi, 2010; Maulana, Hikmah, Shita, Permatasari, & Widyarti, 2014; Mena Laura et al., 2019; Plut et al., 2015; Santamaria-Jr et al., 2019; Sun et al., 2017; Villarino et al., 2011; Zhang, Li, & Bi, 2011).

Study design and characteristics

All study characteristics and qualitative data are presented in Table 1.

Half of the included studies (7/14; 50%) originated from South America, ie Brazil or Argentina, while 5 originated from Asia (36%) and 2 from Europe (14%). Publication years ranged from 2010 to 2019 with the preponderance of studies being published within the last 4 years (8/14; 57%).

All but one study (Braga et al., 2011), recruited rats as animal models and specifically Wistar rats or Sprague- Dawley species (Arita et al., 2016; Damanakis, 2018; Ferreira et al., 2018; Gomes et al., 2017, 2018; Li et al., 2010; Maulana et al., 2014; Mena Laura et al., 2019; Plut et al., 2015; Santamaria-Jr et al., 2019; Sun et al., 2017; Villarino et al., 2011; Zhang et al., 2011). Braga et al, (2011) (Braga et al., 2011) recruited mice. Male rats were used with an age range from 4 to 14 weeks. For the majority of studies, diabetes status induction was achieved through the injection of variable concentrations of streptozotocin (9/14; 64%) ranging from 20 to 120 mg/kg. As an alternative, 4 studies used alloxane monohydrate in a concentration ranging from 40 to 150 mg/kg in a sterile saline solution, while only one study used an established model for non- obese type- II diabetes (Plut et al., 2015). Planned initial sample sizes ranged from 16 to 100 animals in total across the studies, with further breakdowns according to the formulated groups. Almost all studies used coil springs between molars and incisors of the animals to induce tooth movement. One used a helicoid spring, connecting the two contralateral molars (Villarino et al., 2011). Timescale for orthodontic force ranged from 8 to 42 days, for outcomes related to tooth movement and related conditions, while for biochemical analysis related outcomes 12 to 72 hours usually sufficed. According to the intended outcomes, the included studies used various methods of assessment, namely radiographs, casts, microcomputed tomography, histological, histomorhometric and immunohistochemical analysis and also gene expression and protein quantification.

Risk of Bias within Studies

The risk of bias of the included in- vivo animal studies was explored through the SYRCLE tool (Figure 2) (Hooijmans et al., 2014). In particular, 9 of 14 studies were rated as unclear risk of bias overall,

leaving 5 studies presenting high risk of bias. Items that mostly contributed to the inspected high risk of bias were related to selection bias (sequence generation, baseline characteristics or allocation concealment). There was no definite information for any or the included studies as to whether housing of animals was done at random or otherwise, while there was also no reporting of blinding of investigators, caregivers or even outcome assessors, rendering the risk of bias status unclear for these domains. Three studies reported experience of animal loss, while all pre-determined outcomes were reported in the results section of the included studies; however no research protocol could be identified for any of the included studies.

Effects of Interventions, Meta-analyses, Additional Analyses

In total, nine articles contributed to meta-analyses (Arita et al., 2016; Damanakis, 2018; Ferreira et al., 2018; Gomes et al., 2017, 2018; Mena Laura et al., 2019; Santamaria-Jr et al., 2019; Sun et al., 2017; Villarino et al., 2011), while there was a variable amount of outcomes across the included studies. Decision for mathematical synthesis of studies was made after consideration of the apparent homogeneity of the eligible studies. First, identification of similar outcomes was done; subsequently, consideration of experimental settings, populations and applied interventions was employed in an attempt to identify comparable and homogenous conditions.

Table 2 presents the quantitative data from meta-analyses as well as from individual study findings.

Specifically, when tooth movement was considered, there was no evidence of a significant effect of diabetes status on the overall orthodontically induced movement (6 studies, SMD: 1.47; 95% CI: -0.60, 3.53; p=0.16; Figure 3), with an increased amount of heterogeneity documented across the contributing studies. Similarly, the concentration of osteoclast cells in the compression sites did not show any difference between diabetic and control animals under mechanical stress (3 studies, SMD: 1.35; 95%CI: -3.40, 6.10; P=0.58; Figure 4). However, evidence on key marker genes of osteogenesis such as osteopontin (OPN), as represented by immunoreactive cells for anti- OPN recorded 21 days after initiation of tooth movement, revealed a significantly lower concentration for the diabetic animals (2 studies, SMD: -3.77; 95%CI: -4.89, -2.66; p<0.001; Figure 5). This was also confirmed by a single study (Sun et al., 2017), that reported suppress of the differentiation associated increase in alkaline phosphatase (ALP) expression when diabetic status was confirmed (1 study, MD: -0.09; 95%CI: -0.10, -0.08; P<0.001).

Further insights within this individual study showed evidence of increase in the cathepsin (CK) expression levels within compression sites of diabetic animals (MD: 0.18; 95%CI: 0.15, 0.21; p<0.001), as well as in the sclerostin (SOST) levels in tension sites (MD: 12; 95%CI: 10.57, 13.43; p<0.001). Other immunohistochemical findings revealed a significant decrease in dentin matrix protein- 1 cells (DMP-1) within tension sites of diabetic animals (MD: -17; 95%CI: -18.51, -15.49; p<0.001).

Moreover, data from an individual study (Santamaria-Jr et al., 2019) revealed a significant increase in a number of protein inflammatory markers in the alveolar bone of diabetic rats. These included the fibroblastic growth factor bFGF (MD: 91.3; 95%CI: 72.46, 110.14; p<0.001), the transforming growth factor TGF-β1 (MD: 96.05; 95%CI: 83.86, 108.24; p<0.001) and the vascular endothelial growth factor VEGF (MD: 68.7; 95%CI: 57.03, 80.37; P<0.001). Findings from tension sites of the periodontal ligament of the animals after orthodontic tooth movement showed a significant decrease in the amounts of fibroblast cells in the diabetic group (MD: -6.10; 95%CI: -8.39, -3.81; p<0.001), accompanied by a significant increase in the inflammatory cells (MD: 3; 95%CI: 1.04, 4.96; p=0.003). Regarding root resorption, only one study (Arita et al., 2016) reported on 2 related outcomes. First, on overall root resorption area, where a significantly lower amount of area crater were detected for the diabetic animals (MD: $-18.7x10^4$; 95%CI: $-24.45x10^4$, $-12.95x10^4$; p<0.001). Second, on total root resorption volume, where in line with the previous finding, a significantly lower amount of resorption was recorded for the diseased animals (MD: $-9.8x10^6$; 95%CI: $-14.65x10^6$, $-4.95x10^6$; p<0.001).

Pre- planned analysis to substantiate the robustness of the retrieved findings after excluding high risk of bias studies was only applicable for the outcome tooth movement. After excluding the study of Santamaria et al., 2019 (Santamaria-Jr et al., 2019) due to the inspection of high risk of bias in more than 1 domains, again, no significant effects were detected in the pooled estimate (5 studies, SMD: 2.30; 95%CI: -0.15, 4.74; p=0.07; Supplementary Figure 1). We further proceeded with a sensitivity analysis excluding the study of Damanakis, 2018 (Damanakis, 2018), after identifying a very low value of standard deviation of the data indicating extremely low variability compared with similar studies. Verification of this recording through communication with authors were confirmatory. In essence, after excluding this study, the overall pooled estimate remained non-significant (5 studies, SMD: 0.61; 95%CI: -1.43, 2.64; p=0.56; Supplementary Figure 2).

On an exploratory basis we examined the effect of diabetic versus insulin- treated diabetic animals. We found no evidence to suggest a difference between insulin- treated and uncontrolled diabetes animals in the recorded tooth movement after orthodontic induction (SMD: -0.14; 95%CI: -1.84, 1.56; p=0.87; Supplementary Figure 3).

Last, pre-planned analyses to explore publication bias and small study effects were ultimately not attempted due to the limited data and the small number of studies contributing to the quantitative syntheses.

Discussion

Summary of the Evidence

This systematic review was conducted and reported based on pre- clinical animal research in view of the lack of clinical studies in human. The findings reflect a wide spectrum of reported outcomes within the included studies. This resulted in a limited amount of data being synthesized to achieve increased precision in the reporting of the estimated effect for specific outcomes. The most represented was the reporting of tooth movement under conditions of health and disease with uncontrolled diabetic status. The null hypothesis was partially rejected. It was evident, that the inferences made for the effect of uncontrolled diabetes on orthodontic tooth movement were merely limited to the documented evidence of no significant effect macroscopically. Only one meta-analysis comprising of two studies was indicative of a decreased tendency for osteoblastic differentiation, related to the key marker gene of osteopontin when diabetic status was considered. Although the findings of both studies (Gomes et al., 2017, 2018) were indicative for a similar direction of the effect, the overall pooled estimate should be interpreted with caution, as these studies might have shared an experimental group; efforts were made to communicate with authors, however, they were unsuccessful.

To our knowledge this is the first systematic review with quantitative syntheses on mechanical stimulation of teeth targeting to subsequent tooth movement, under hyperglycemic metabolic conditions and compared to the physiologic normoglycemic status. Although our initial intention was to search for and document any clinical trial or prospective/ retrospective epidemiologic study with the involvement of patients or patient records of orthodontically treated individuals under the metabolic condition of diabetes, this was ultimately not applicable. There is a gap in the existing

literature between human and animal research characterized by complete lack of evidence emerging from clinical research (Almadih et al., 2018; Burden et al., 2001), as no study could be identified except for isolated case reports (Maia, Monini, Jacob, & Gandini, 2011; Reichert, Deschner, & Jäger, 2009) while animal research has been identified as the sole source of evidence (Holtgrave, & Donath, 1989; Najeeb et al., 2017).

Current reporting from basic research suggests that hyperglycemic environment may compromise connective tissue homeostasis and remodeling of the implicated structures (Guo, Chen, Zhang, Ding, & Wang, 2018; Kato et al., 2016; Papadopoulou, Torado, Eliades, & Kletsas, 2019). A laboratory study on human periodontal ligament (PDL) fibroblasts, being subjected to high glucose concentration under mechanical stretching conditions, has revealed the capacity of the simulated diabetic status to inhibit the documented effects of tensile stretching with regard to osteogenic differentiation potential, if applied in isolation (Papadopoulou et al., 2019). More specifically, PDL cells stretched under conditions of hyperglycemia have shown downregulation or attenuation of key genes or transcriptional factors such as osteopontin (OPN), alkaline phosphatase (ALP) and c-fos. The results of one quantitative synthesis from the present review confirmed these findings for OPN, while for ALP, there was evidence in the same direction from an individual study, as no additional research findings were identified for this outcome. This is effectively important for patients undergoing orthodontic treatment, with compromised metabolism, as is the case with poorly controlled diabetes mellitus. In such patients who bear a challenging homeostasis, it might be inferred that loading conditions within the periodontal ligament, under the mechanisms of tooth movement, may be deteriorating for the tissue itself. It has also been claimed, that the effect of diabetes on stretch- induced PDL response might be partially attributed to the increased osmolality of the tissue which appears as a significant regulator (Mavrogonatou, & Kletsas, 2012; Papadopoulou et al., 2019).

Furthermore, additional evidence from the retrieved studies at the histological and immunohistochemical level, reveal an increased amount of osteoclast cells within the periodontal ligament of the compressed tooth sites coupled with elevated cathepsin levels, aligned with augmented sclerostin levels within the tension sites (Sun et al., 2017). These findings present a possible potential of the diabetic status to dictate uncontrolled and excessive tooth movement, overshadowing the physiologic amounts, even when the periodontal apparatus appears intact. To the same line and through the use of molecular analytical techniques and protein quantification

procedures, inflammatory markers as represented by endothelial or fibroblastic growth factors of the alveolar bone have been shown to increase their levels. This might be a reactive activity following the hampered organization of the collagen fiber network (Santamaria-Jr et al., 2019; Zhang et al., 2011).

Although significant alterations have been recorded at the molecular, or immunohistological level, the clear picture does not reveal itself macroscopically, if one looks at the amount of tooth movement in terms of diabetic and normoglycemic status. Evidence form the quantitative synthesis of six studies on tooth movement showed null effect. It is interesting that of the six studies combined, only one revealed no difference (Ferreira et al., 2018). Three of the studies (Damanakis, 2018; Gomes et al., 2018; Mena Laura et al., 2019) reported a significant effect of hyperglycemic status to promote increased tooth movement, while the other two revealed completely opposite findings (Arita et al., 2016; Santamaria-Jr et al., 2019). Only one of those studies assessed the magnitude of root resorption at the end of the corresponding tooth movement (Arita et al., 2016). Not surprisingly, for this specific study, this outcome was coupled with the reported findings on overall tooth movement, where the hyperglycemic conditions seemed to impose an attenuation in the amount of both movement and resorption. However, evidence from other studies is lacking. Possible explanations for the above- mentioned discrepancies between the studies are: differences in the induction of the hyperglycemic status or the notion of the levels of glucose that could be defined as excessive and corresponding to diabetes mellitus per se; limited timescale for tooth movement, pertaining to small amounts of movement; differences in settings, laboratory conditions or population samples used in each individual study. The last could also be confirmed by the apparent heterogeneity identified. With regard to the central outcome of tooth movement, as indicated by our exploratory analysis, we did not identify any difference between diabetic and insulin- treated animal populations, effectively confirming the findings of the aforementioned primary analysis and substantiating the potential for a limited experimental duration.

Strengths and Limitations

This review presents several strengths, being the first to include a quantitative synthesis of quite a few outcomes related to tooth movement under the condition of diabetes mellitus, resulting in increased precision of the estimated effect compared to data from individual single studies. Electronic search was extensive within eight databases of published and unpublished research and

we followed a clear and transparent methodology in conducting and reporting the present systematic review. In addition, the protocol of this review was registered a priori to avoid significant deviations that could allow for selective reporting and its implications (Fleming, Koletsi, Dwan, & Pandis, 2015).

However, some limitations also exist. The quantitative syntheses were actually employed but based on a limited number of studies; only for the outcome tooth movement there were 6 contributing studies and even in this synthesis it was not possible to search for and detect publication bias that might have a bearing on the retrieved findings. There may be a conceivable lack of complete certainty with regard to the evidence provided, as the individual studies identified and ultimately included, were not free from methodological limitations and serious risk of bias issues. Extrapolation to human related conditions should be avoided in a direct manner, as there is a number of technical uncertainties and/ or experimental/ clinical conditions that are not to be translated to clinical practice; some examples are duration and timing of orthodontic treatment, orthodontic tooth movement under complete and uncontrolled hyperglycemic condition, as well as excessive force magnitude applications (Li et al., 2010; Sun et al., 2017; Theodorou, Kuijpers-Jagtman, Bronkhorst, & Wagener, 2019; Zhang et al., 2011). In addition, although we attempted to contact the authors of the studies in order to augment data contribution to the quantitative syntheses, this was actually not achieved, except in one case, due to the perceived reluctance of the authors to respond. Lack of standardization of laboratory settings and conditions related to diabetes induction, tooth movement, duration of experiments might have also contributed to the overall high levels of the documented heterogeneity across studies (Clarke, & Williamson, 2016). Last, search strategy was implemented in a manner that would allow for an effectively wide initial inclusion of relevant articles under the spectrum of any article related to “tooth movement” and “diabetes”. The specific inclusion of the term “root resorption” as an additional outcome term in the search slot was also prioritized, based on the fact that resorption is recognized as an important adverse outcome potentially related to orthodontic treatment and tooth movement in particular, and orthodontic tooth movement is frequently studied as a potential prognostic factor for root resorption (Currell, Liaw, Blackmore Grant, Esterman, & Nimmo 2019; Weltman, Vig, Fields, Shanker, & Kaizar, 2010). In any case, the inclusion of the term “root resorption” under the Boolean operator “OR” within the search strategy, is unlikely that would have resulted in bias or non-inclusion of other outcome- specific articles.

Conclusions

According the findings of this review and based on evidence from pre- clinical *in vivo* research, presence of hyperglycemia and background of diabetes mellitus is a regulator of immunohistochemical and inflammatory alterations within the periodontal ligament of teeth and supporting alveolar apparatus. Under mechanical stimulation, as represented by orthodontic tooth movement, this effect seems to be augmented towards the direction of attenuated osteoblastic differentiation; however, findings at the macroscopic level were not confirmatory in their entirety.

In all, this is a field with complete lack of evidence form human research and any new effort should be directed towards exploring the effects of the human disease on tooth- bone interactions after mechanical stimulation. Otherwise, clinical practice and decisions for metabolically compromised patients proceeding for orthodontic treatment shall only be based on expert opinions and being grounded away from evidence.

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Conflict of Interest

The authors have nothing to declare

Appendix A. Search Strategy for study selection

No.	Electronic Database	Hits
1.	**Medline via Pubmed**	
	((diabetes) OR (diabetic) OR (diabetes mellitus)) AND ((tooth movement) OR (orthodontic) OR (orthodontic movement) OR (root resorption) OR (root remodeling))	**111**
2.	**Scopus**	
	((diabetes) OR (diabetic) OR (diabetes mellitus)) AND ((tooth movement) OR (orthodontic) OR (orthodontic movement) OR (root resorption) OR (root remodeling))	**135**
3.	**Cochrane Central Register of Controlled Trials (CENTRAL)**	
	((diabetes) OR (diabetic) OR (diabetes mellitus)) AND ((tooth movement) OR (orthodontic) OR (orthodontic movement) OR (root resorption) OR (root remodeling))	**28**
4.	**Cochrane Database of Systematic Reviews (CDSR)**	
	((diabetes) OR (diabetic) OR (diabetes mellitus)) AND ((tooth movement) OR (orthodontic) OR (orthodontic movement) OR (root resorption) OR (root remodeling))	**0**
5.	**Open Grey**	
	(diabetes) AND (tooth movement)	**0**
	(diabetes) AND (orthodontic)	**0**
6.	**ClinicalTrials.gov (www.clinicaltrials.gov)**	
	(diabetes) AND (tooth movement)	**0**
	(diabetes) AND (orthodontic)	**0**
7.	**National Research Register (ISRCTN: www.controlled-trials.com)**	
	(diabetes) AND (tooth movement)	**3**
	(diabetes) AND (orthodontic)	**10**
8.	**PERGAMOS (pergamos.lib.uoa.gr)**	
	(diabetes) AND (tooth movement)	**1**

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FIGURE CAPTIONS

Figure 1. Flow diagram of study selection.

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study. The green plus sign indicates low risk of bias; the red minus indicates high risk of bias while the yellow question mark shows unclear risk of bias domains.

Figure 3. Random effects meta-analysis for the standardized mean difference in tooth movement induced by orthodontic forces between diabetic and normoglycemic rats.

Figure 4. Random effects meta-analysis for the standardized mean difference in the amounts of osteoclast cells within the compression sites of the periodontal ligament between diabetic and normoglycemic rats, after application of orthodontic forces.

Figure 5. Random effects meta-analysis for the standardized mean difference in the concentration of immunoreactive cells for anti- osteopontin (OPN) recorded 21 days after the initiation of tooth movement within the compression sites of the periodontal ligament between diabetic and normoglycemic rats.

TABLES

Table 1. Characteristics of included studies (n=14).

Author	Sample	Interventions	Outcomes	Method of outcome assessment	Timescale for orthodontic force	Diabetes induction
Arita et al., 2016 (Japan)	N=23, age: 10 weeks, male Sprague-Dawley rats	A nickel–titanium closed-coil spring of 10 g was applied for 2 weeks to the maxillary left first molar in all rats to induce mesial tooth movement. Intervention A: control normoglycemic (n=7), Intervention B: diabetic (n=9), Interventions C: diabetic+ insulin (n=7)	1. tooth movement, 2. tooth inclination, 3. root resorption	microcomputed tomography images	14 days (2 weeks after STZ injection)	a single intraperitoneal injection of streptozotocin (STZ) 60 mg/kg
Braga et al., 2011 (Brazil)	N=60, age: 10 weeks, male C57BL6/J mice	An orthodontic appliance consisting of a nickel-titanium (Ni-Ti) 0.25 · 0.76 mm coil spring, bonded between the maxillary right first molar and the incisors and 35g force in the mesial direction. Intervention A: control normoglycemic (n=25), Intervention B: diabetic (n=25), Interventions C: diabetic+ insulin (n=10)	1. tooth movement, 2. TRAP- positive osteoclasts, 3. cytokine and chemokine expression, 4. osteoblastic and osteoclastic markers (Alp, Col1, Runx2, Ocn)	microscope and digital camera, real- time PCR	6 and 12 d for histological measurements; and 0, 12, and 72 h for biochemical analyses.	intraperitoneal injection of 120 mg/ kg of streptozotocin

Damanakis (thesis), 2018 (Greece)	N=16, age: 4 weeks, male Wistar rats	A closed coil spring of 30g was applied for 2 weeks to the maxillary right first molar in all rats to induce mesial tooth movement. Intervention A: control normoglycemic (n=8), Intervention B: diabetic (n=8)	1. histologic and histochemical outcomes, 2. tooth movement	radiographs, casts, digital analysis of geometric image characteristics	21 days (1 week after SZT injection)	a single intraperitoneal injection of streptozotocin 60 mg/kg
Ferreira et al., 2018 (Brazil)	N=40, age: 13 weeks, male Wistar rats	A 4-mm closed-coil spring made of CrNi connected molars to incisors to induce orthodontic tooth movement in groups with tooth movement. Intervention A: control normoglycemic w/o tooth movement, Intervention B: control normoglycemic with tooth movement (OM), Intervention C: control normoglycemic with ligature induced periodontitis (P), Intervention D: control normoglycemic with OM and P; Intervention E: diabetic, Intervention F: diabetic+ OM, Intervention G: diabetic+ P, Intervention H: diabetic+ OTM+ P (n=5 each)	1. tooth movement, 2. histologic outcomes (bone mineral density)	digital caliper, histologic analyses	8 days (after initial 4 weeks for diabetes induction plus 1 week for periodontal disease induction)	intraperitoneally with alloxan monohydrate in a sterile saline solution at a concentration of 150 mg/kg

Gomes et al., 2017 (Brazil)	N=60, age: adult, male, Wistar rats	1. A 4-mm closed-coil spring made of NiTi connected molars to incisors with a force magnitude of 20cN to induce orthodontic tooth movement 2. diode laser emission of 780nm wavelength, output power of 20mW, energy density of 640J/cm2 for 40s on the middle third of the root of the first molar. Intervention groups N: control normoglycemic, LN laser-normoglycemic, D diabetic, LD laser-diabetic Each group was euthanized at 7,14 and 21 days after appliance installation (n=5)	1. histomorphological outcomes, 2.immunohistochemical analysis (OPN,OPG,RANKL)	histologic analysis, immunohistochemical analysis	7 days after diabetes was confirmed the appliance was place remained from 7 up to 21 days	intraperitoneally with alloxan monohydrate at a concentration of 40 mg/kg

Gomes et al., 2018 (Brazil)	N=40, age: adult(90-100 days), male, Wistar rats	1.A 4-mm closed-coil spring made of NiTi connected molars to incisors with a force magnitude of 20cN to induce orthodontic tooth movement 2. diode laser emission of 780nm wavelength, output power of 20mW, energy density of 160, 320, 640J/cm2 for 10,20, 40s on the middle third of the root of the first molar. Each animal received 10 doses of radiation N normoglycemic, 160 J-LN 160 J-laser-normoglycemic, 320 J-LN 320 J-laser-normoglycemic, 640 J-LN640 J-laser-normoglycemic, D diabetic, 160 J-LD 160 J-laser-diabetic, 320 J-LD 320 J-laser-diabetic, 640 J-LD 640 J-laser-diabetic n=5	1. tooth movement, 2. histomorphological analysis, 3. immunohistochemical analysis (OPN)	radiographs, histologic analysis, immunohistochemical analysis	placed 7 days after diabetes was confirmed, 21 days	intraperitoneally with alloxan monohydrate at a concentration of 40 mg/kg
Li et al., 2010 (China)	N=48, age:7weeks, male, Sprague-Dawley rats	1. NiTi coil force level 0.5N between molars and incisors "normal" n=24 "diabetes" n=24 the rats were euthanized 3, 7, and 14 days after orthodontic induction.	1. histological analysis, 2.immunohistochemical analysis	Col-I and Col-III fibers, expression of Col-I, MMp-1 and TIMP-1, osteoclasts count	3,7 and 14 days (8 weeks after diabetes was induced)	single intraperitoneal injection of STZ 65mg/kg

Maulana et al., 2014 (Indonesia)	N=24, age: 4 month old, male, Wistar rats	1. Orthodontic appliance bonded to the upper incisors force magnitude 10,20,30 grF K1 (normal, without orthodontic appliances), K2 (diabetes, without orthodontic appliances), K3 (normal, with orthodontic appliance 30 grF), K4 (diabetes, with orthodontic appliance 10 grF), K5 (diabetes, with orthodontic appliance 20 grF), and K6 (diabetes, with orthodontic appliance 30 grF)	1. tooth movement, 2. histomorphological analysis	digital caliper, histologic analyses	7 days	imtraperitoneally with Streptozotocin stratified dose (STZ-SD) of 40,35,30,25,20 mg/kg for 5 consecutive days
Mena Laura et al., 2019 (Brazil)	N=100, age: 8 week old, male, Wistar rats	1. A 9-mm NiTi closed coil spring between molars and incisors NG (n=20) control non diabetic T1D diabetic (n=20) treated with saline solution I-T1D (n=20) 2 IU slow-release insulin in the morning and 2 IU of regular plus 2 IU of slow-release insulin in the evening. Total 6IU IM-T1D (n=20) treated as I-T1D plus 150mg/kg of metformin	1. tooth movement, 2. histological analysis, 3. immunohistochemical analysis	micro-CT, histologic analysis, immunohistochemical analysis	14 days (2 weeks after STZ injection)	single intraperitoneal injection of 47 mg/kg of streptozotocin

Plut et al., 2015 (Slovenia)	N=14, age: 13-14weeks, male, Wistar rats, N=14, age: 13-14weeks, male, GK rats	1. superelastic closed coil spring (25cN)between molars and incisors. Wistar control group (n =8) GK control group (n =8) Wistar appliance group (n =16) GK appliance group (n =16)	1. tooth movement, 2.histomorphometric analysis, 3.gene expression	digital caliper, histologic analyses, gene expression	42 days	spontaneous T2D Goto-Kakizaki (GK)
Santamaria Jr et al., 2019 (Brazil)	N=40, age: 90 days, male, Wistar rats	1.ligature- induced periodontitis 2.SS spring extended 2mm (10 days, 0.4 N force); OTM: orthodontic movement, P + OTM: periodontitis and orthodontic movement, D + OTM: diabetes and orthodontic movement, D + P + OTM: diabetes, periodontitis and orthodontic movement (n=10 per group)	1. tooth movement, 2. histomorphometric analysis, 3. biochemical analysis	digital caliper, histomorphometric analysis, protein quantification in the gingival tissue and alveolar bone	10 days (60 days after diabetes induction)	Alloxan-induced diabetes, 150 mg/kg
Sun et al., 2016 (China)	N=30, age: 7 weeks, male, Wistar rats	1. intragastric administration of metformin 100mg/kg every day for 1 month 2.coil NiTi 0.012inch force of 0.5N. (DB) type 2 diabetes group, n=10; (MA) metformin group, n=10; (NG) normoglycemic n=10	1. tooth movement, 2. histological analysis, 3. immunohistochemical analysis	digital microscope, gene expression, osteoclast count, alkaline phosphatase (ALP), cathepsin K (CK), sclerostin (SOST), dentin matrix protein 1 (DMP-1)	14 days	single intraperiotoneal injection of STZ 35mg/kg

Villarino et al., 2011 (Argentina)	N=24,male, Wistar rats	1. 0.014-in circular cross-section SS wire shaped into a helical spring force magnitude of 120 +/-15 g toward the vestibular plate 2. daily administration of human NPH insulin Experimental orthodontics (ORT) Experimental diabetes and orthodontics (DBT+ORT) Experimental diabetes treated with insulin and orthodontics (DBT 1 INS 1 ORT) (n=8 per group)	1. body weight, 2. histological analysis, 3. histomorphometric analysis	digital laboratory balance Ohaus, histomorphometric analysis (bone activity, number of osteoclasts, bone volume of the interradicular bone)	48h (placed 6 weeks after diabetes was induced)	single intraperitoneal injection of streptozotocin (STZ) 60 mg/kg
Zhang et al., 2011 (China)	N=48, age: 6-7 weeks, male, Sprague-Dawley rats	1. NiTi coil between upper incisor and upper 1st molar force level approx 50g nondiabetic (ND) diabetes induced (DI) (n=24 per group)	1. histological analysis, 2.immunoreactivity of collagen-I, MMP-1, TIMP-1	histological analysis, immunohistochemistry	4 rats in each group (ND,DI) were killed at 1, 3, 5, 7, 10 and 14 d after appliance placement (tooth movement 8 weeks after diabetes was induced)	single intraperitoneal injection of 65 mg/kg streptozotocin

Table 2. Quantitative data from meta-analyses and individual single studies for related outcomes (Diabetic vs Normoglycemic). The minus sign (-) shows lower effect for the diabetic group. Bold indicate statistically significant comparisons.

#	Study ID	Outcome	MD or SMD (95% CIs)	P-value	Heterogeneity (I^2%)
1	6 studies	Tooth movement	SMD: 1.47 (-0.60, 3.53)	0.16	92
2	3 studies	Osteoclast cells (compression site)	SMD: 1.35 (-3.40, 6.10)	0.58	96
3	2 studies	Immunoreactive cells for anti- OPN (21 days)	SMD: -3.77 (-4.89, -2.66)	**<0.001**	0
4	1 study	Bone Loss (mm^2)	MD: 0.06 (-0.01, 0.13)	0.08	-
		Bone Density (%)	MD: -4.5 (-14.30, 5.30)	0.37	-
5	1 study	Root Resorption Area (10^4 μm^2)	MD: -18.7 (-24.45, -12.95)	**<0.001**	-
		Root Resorption Volume (10^6 μm^3)	MD: -9.8 (-14.65, -4.95)	**<0.001**	-
6	1 study	Immunoreactive cells for anti- OPG (21 days)	MD: -0.77 (-1.11, -0.43)	**<0.001**	-
		Immunoreactive cells for anti- RANKL (21 days)	MD: -0.07 (-0.27, 0.13)	0.49	-
7	1 study	ALP expression (tension site)	MD: -0.09 (-0.10, -0.08)	**<0.001**	-
		CK expression (compression site)	MD: 0.18 (0.15, 0.21)	**<0.001**	-
		SOST (cells/ mm^2) (tension site)	MD: 12 (10.57, 13.43)	**<0.001**	-
		DMP- 1 (cells/ mm^2) (tension site)	MD: -17 (-18.51, -15.49)	**<0.001**	-
8	1 study	bFGF*	MD: 91.3 (72.46, 110.14)	**<0.001**	-
		TGF-β1*	MD: 96.05 (83.86, 108.24)	**<0.001**	-
		VEGF*	MD: 68.7 (57.03, 80.37)	**<0.001**	-
		Fibroblast cells (n/10^4 μm^2) (tension site)	MD: -6.10 (-8.39, -3.81)	**<0.001**	-
		Inflammatory cells (n/10^4 μm^2) (tension site)	MD: 3 (1.04, 4.96)	**0.003**	-
		Blood vessels (n/10^4 μm^2) (tension site)	MD: -0.4 (-1.06, 0.26)	0.23	-

MD, mean difference; SMD, standardized mean difference; OPN, osteopontin; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor kappa B ligand; ALP: alkaline phosphatase; CK, cathepsin K; SOST, sclerostin; DMP- 1, dentin matrix protein 1; bFGF, fibroblastic growth factor; TGF-β1, transforming growth factor; VEGF, vascular endothelial growth factor
*protein quantification in the alveolar bone (inflammatory markers)

FIGURES

Figure 1.

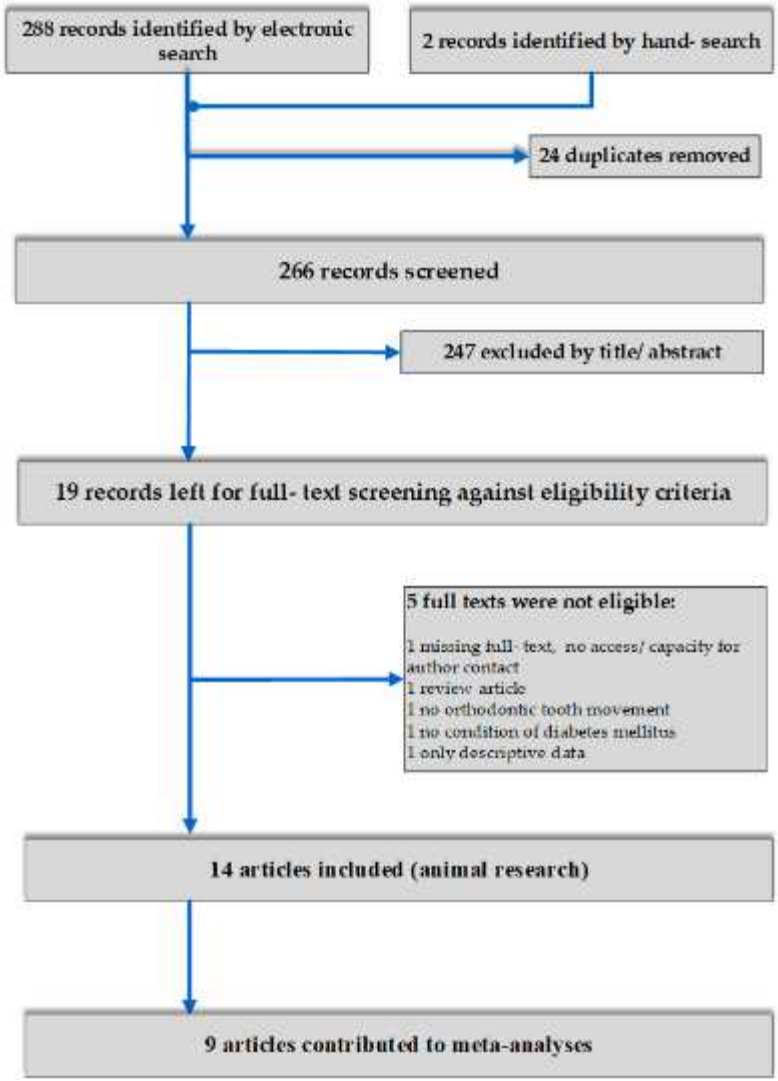


Figure 2.

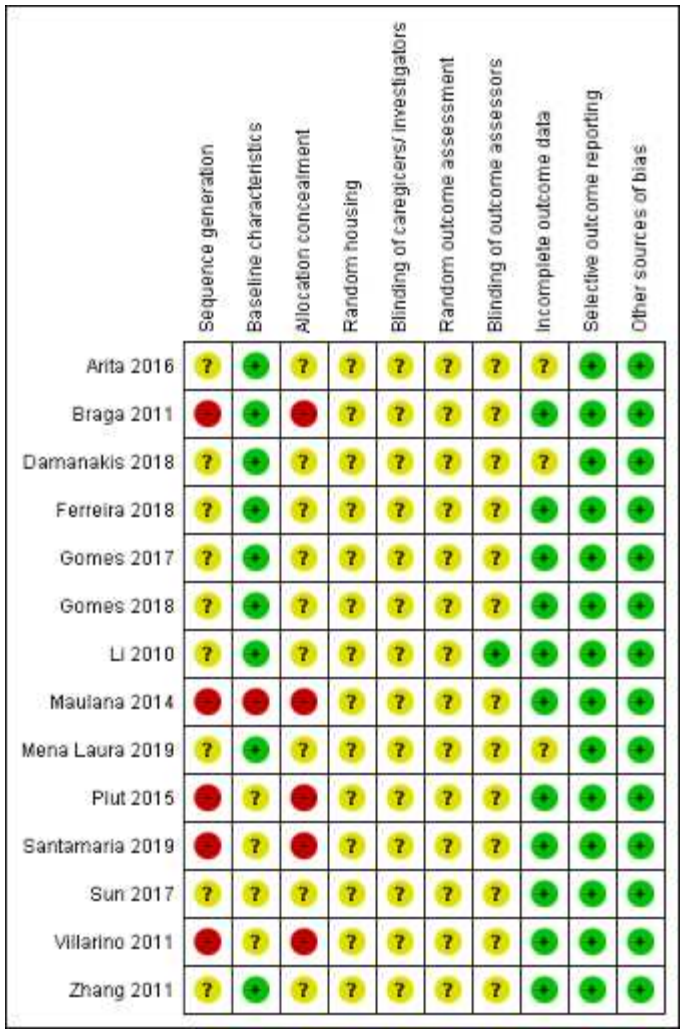
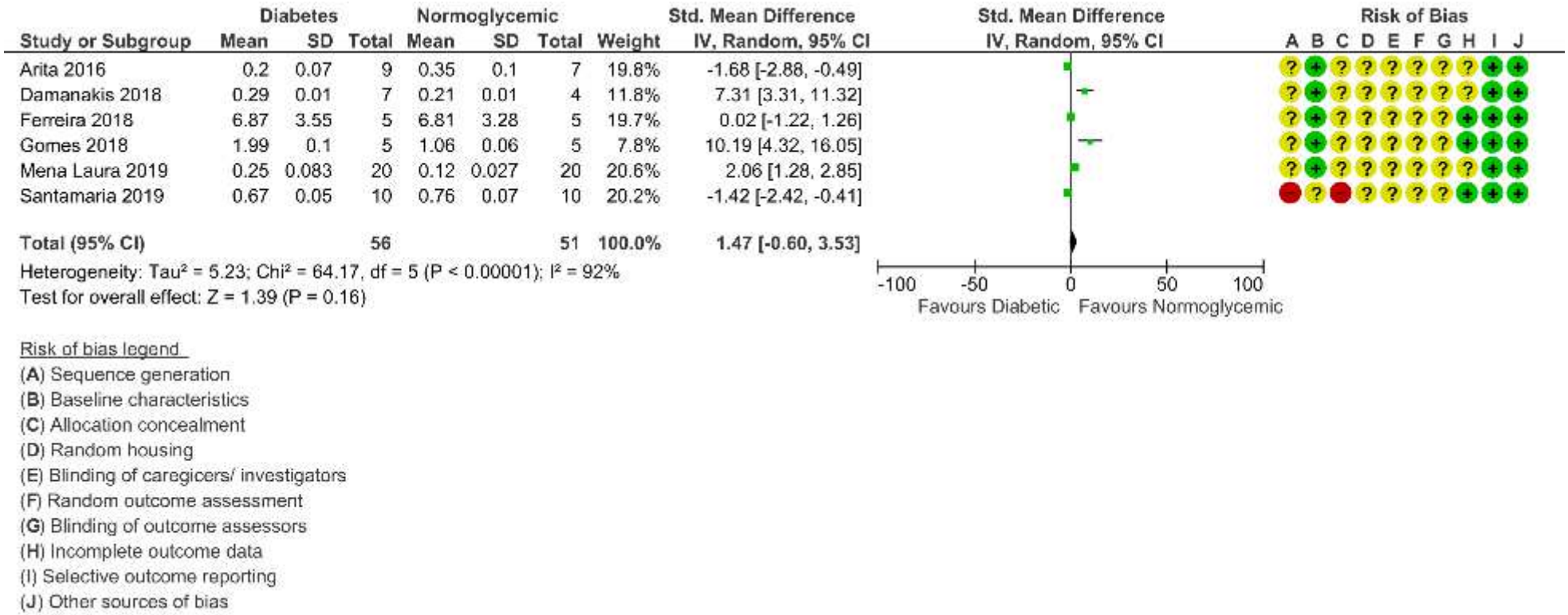

Sequence generation
Baseline characteristics
Allocation concealment
Random housing
Blinding of caregicers/ investigators
Random outcome assessment
Blinding of outcome assessors
Incomplete outcome data
Selective outcome reporting
Other sources of bias
Arita 2016
Braga 2011
Damanakis 2018
Ferreira 2018
Gomes 2017
Gomes 2018
Li 2010
Maulana 2014
Mena Laura 2019
Plut 2015
Santamaria 2019
Sun 2017
Villarino 2011
Zhang 2011

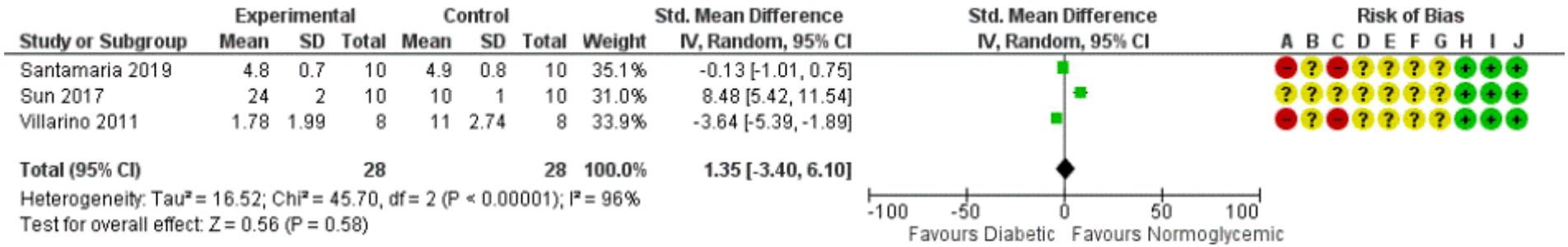
Figure 3.



Risk of bias legend

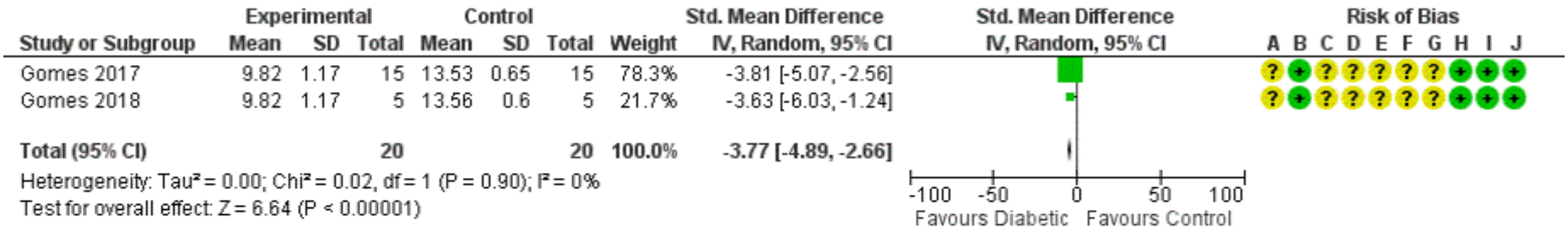
(**A**) Sequence generation
(**B**) Baseline characteristics
(**C**) Allocation concealment
(**D**) Random housing
(**E**) Blinding of caregicers/ investigators
(**F**) Random outcome assessment
(**G**) Blinding of outcome assessors
(**H**) Incomplete outcome data
(**I**) Selective outcome reporting
(**J**) Other sources of bias

Figure 4.



Risk of bias legend
(A) Sequence generation
(B) Baseline characteristics
(C) Allocation concealment
(D) Random housing
(E) Blinding of caregicers/ investigators
(F) Random outcome assessment
(G) Blinding of outcome assessors
(H) Incomplete outcome data
(I) Selective outcome reporting
(J) Other sources of bias

Figure 5.



Risk of bias legend

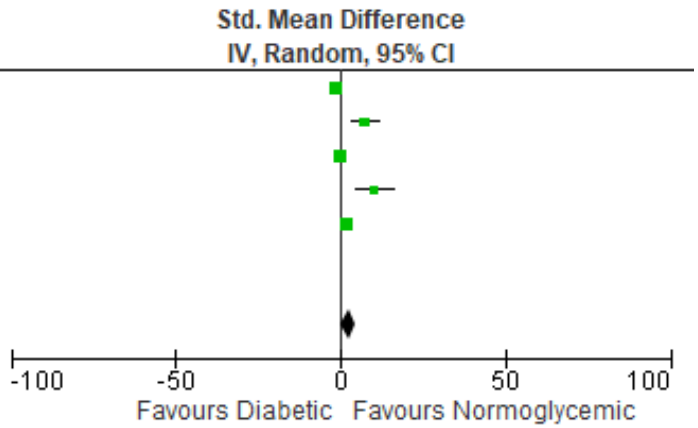
(**A**) Sequence generation
(**B**) Baseline characteristics
(**C**) Allocation concealment
(**D**) Random housing
(**E**) Blinding of caregicers/ investigators
(**F**) Random outcome assessment
(**G**) Blinding of outcome assessors
(**H**) Incomplete outcome data
(**I**) Selective outcome reporting
(**J**) Other sources of bias

SUPPLEMENTARY FILES

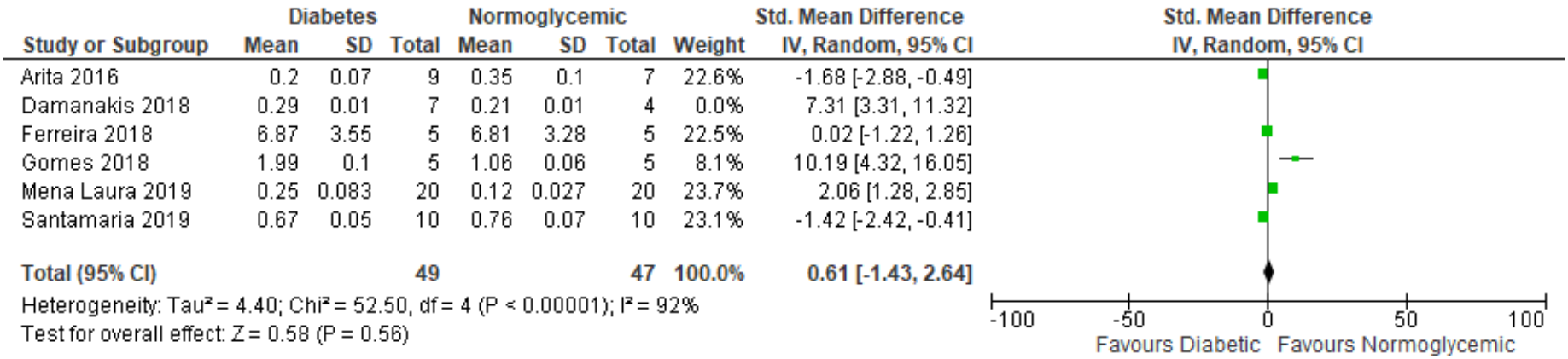
Supplementary Figure 1. Sensitivity analysis for the standardized mean difference in tooth movement induced by orthodontic forces between diabetic and normoglycemic rats, after excluding the study Santamaria et al., 2019 due to the inspection of high risk of bias.

	Diabetes			Normoglycemic				Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Arita 2016	0.2	0.07	9	0.35	0.1	7	24.5%	-1.68 [-2.88, -0.49]
Damanakis 2018	0.29	0.01	7	0.21	0.01	4	15.3%	7.31 [3.31, 11.32]
Ferreira 2018	6.87	3.55	5	6.81	3.28	5	24.4%	0.02 [-1.22, 1.26]
Gomes 2018	1.99	0.1	5	1.06	0.06	5	10.4%	10.19 [4.32, 16.05]
Mena Laura 2019	0.25	0.083	20	0.12	0.027	20	25.4%	2.06 [1.28, 2.85]
Santamaria 2019	0.67	0.05	10	0.76	0.07	10	0.0%	-1.42 [-2.42, -0.41]
Total (95% CI)			**46**			**41**	**100.0%**	**2.30 [-0.15, 4.74]**

Heterogeneity: Tau² = 5.98; Chi² = 47.87, df = 4 (P < 0.00001); I² = 92%
Test for overall effect: Z = 1.84 (P = 0.07)



Supplementary Figure 2. Sensitivity analysis for the standardized mean difference in tooth movement induced by orthodontic forces between diabetic and normoglycemic rats, after excluding the study Damanakis 2018 due to the detection of extremely low variability of the treatment effects.



Supplementary Figure 3. Exploratory analysis for the standardized mean difference in tooth movement induced by orthodontic forces between diabetic (experimental) and insulin- treated (control) diabetic rats.